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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
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| 09/869,106 | 10/09/2001 | Isabelle Poquet | 045636-5048 | 1150 |

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| EXAMINER | |
|---|---|
| GRASER, JENNIFER E | |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1645 | |

DATE MAILED: 03/05/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

SUPPLEMENTAL

# Office Action Summary

| Application No. | Applicant(s) | |
|---|---|---|
| 09/869,106 | POQUET ET AL. | |
| **Examiner** | **Art Unit** | |
| Jennifer E. Graser | 1645 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *11 and 13-18* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *11 and 13-18* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *2/24/04* .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

## DETAILED ACTION

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office Action.

Acknowledgment and entry of the Amendment submitted 11/10/03 is made. Claims 11 and 13-18 are currently pending.

### *Claim Rejections - 35 USC § 112*

1. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Claims 11 and 13-18 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 11 and 17 are vague and indefinite because it is unclear which Gram positive bacterial strains are encompassed under the definition "wherein the size of the genome of the bacterial strain is equal to or less than 3.2 Mb". This description is not sufficient to satisfy the Statute's requirement of adequately describing and setting forth the inventive concept. The claim should provide additional properties which would allow for one to identify the bacterial strains which can be used without ambiguity.

Claims 11 and 17 are also vague and indefinite because it is unclear if the claims intend to encompass heterologous or homologous protein expression. The claims read "culturing a Gram positive bacterial strain that expresses said protein". Does this mean only proteins which are homologous to the Gram positive bacterium are to be produced? Do the claims encompass the Gram positive bacterium being used as a transformant to express heterologous proteins or are they intended to encompass solely mutant bacterium which express homologous/native proteins? The current claims appear to read on the latter. If this is not the case, a transformation step should be added to the claimed method. The claim currently reads on recovering protein from naturally occurring Gram positive mutants which do not express functional HtrA protease. Clarification is needed.

Claims 15 and 18 are vague and indefinite because the wording of the claim makes it unclear if the "PtrP protease" mentioned is in addition to the 'HtrA protease' mentioned in the claim from which it depends or if Applicants are referring to the fact that 'PtrP' is a homolog of the 'HtrA' protease. The insertion of the word "also" after the word 'strain' in the second line would clarify the claim.

***Claim Rejections - 35 USC § 112***

3. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. Claims 11 and 13-18 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling 'a method of producing a protein of interest, comprising:, transforming a mutant *L.lactis* bacterium which does not express a functional HtrA protease with a recombinant expression vector comprising a DNA sequence which encodes the protein of interest, culturing said transformed bacterial cell under conditions suitable for gene expression and recovering said protein of interest which is exported by said bacterium in the culture medium' does not reasonably provide enablement for 'a method of producing a protein of interest, comprising:, culturing a Gram positive bacterial strain that expresses the said protein, wherein the size of the genome of the bacterial strain is equal to or less than 3.2 Mb. and wherein the bacterial strain does not express a functional HtrA protease and recovering said protein exported by said strain in the culture medium'. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The present specification teaches that the discovery of the existence of a gene of the *htrA* family in *L.lactis* was made. It is taught that it was found that making a mutation to the *L.lactis* bacterium so that it could no longer produce a functional HtrA protease was sufficient to completely abolish the degradation of the exported proteins. The specification teaches that this was surprising given the residual proteolysis observed previously in other bacteria after inactivation on proteases of the HtrA family. The prior art and the specification teach that mutant E.coli strains in which the gene encoding the HtrA/DegP protease has been inactivated

does not result in a complete abolishment of the degradation of exported proteins as it does in *L.lactis*. It is taught in the specification and the prior art that a large number of bacterial species have several proteases of the HtrA family and several also have serine proteases which are believed to be related to the HtrA family. Exported proteases which are not related to HtrA have also been demonstrated, such as in *B.subtilis*. However, when mutations to several of the exported proteases in these bacterium were made proteolysis of exported proteins still existed. It is taught in the prior art that most proteins produced by bacteria are degraded by more than one protease. Therefore, the use of mutants deficient in the synthesis of a single enzyme can only partially prevent the degradation of the product. Studies in which more mutations were made to try to further rid the proteolysis effect; however, it was found that accumulation of the mutations affect strain viability which causes a significant decrease in growth rate. See pages 2-3 of the specification. The prior art has established that there is great variability and unpredictability in determining which mutations will completely abolish proteolytic activity. The prior art has also established that there is great variability in the number and types of proteases produced among different Genus and species of bacteria. The specification has only taught and provided results in *L.lactis* with the inactivation of the *htrA* gene. This was an unexpected and surprising finding. Especially since inactivation of the *htrA* gene in *B.subtilis* and *E.coli* did not abolish proteolytic activity. The instant specification is not enabled for the broad scope of invention which covers using any Gram positive bacterium with a genome equal to or less than 3.2Mb and which does not express a functional HtrA protease because only the use of *L.lactis* has been taught. It is

unclear that the inactivation of an *htrA* gene in any other bacterium of this size would have similar results. The prior art has established that it is completely unpredictable to determine when inactivation of *an htrA gene or htrA gene homolog* will completely abolish proteolytic activity. It is also unpredictable to determine how many different proteases are produced by any Genus/species of bacteria. The specification also teaches that the term "HtrA protease" is intended to many any serine protease of the trypsin type, having functional and structural similarities with the HtrA protease of *E.coli* (see page 8, lines 5-11); however, the specification only provides enablement and support for methods using a mutant *L.lactis* bacterium which does not express a functional HtrA protease. Accordingly, given the lack of guidance and working examples provided in the specification, the scope of invention is not enabled. Only prophetic examples are provided for using bacterium of different Genus and/or species in the protein production methods.

***Claim Rejections - 35 USC § 102***

5. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

6. Claims 11, 13, 14, 16 and 17 are rejected under 35 U.S.C. 102(b) as being anticipated by Vos et al (WO 91/02064).

Vos et al teach a method of producing a protein of interest, i.e., casein, comprising culturing a Gram positive bacterial host cell, *Lactobacillus sp.*, which do not express a functional HtrA protease and recovering said protein exported by said strain in the culture medium. The instant specification has defined 'HtrA protease' as 'any serine protease of the trypsin type, having functional and structural similarities with the HtrA protease of *E.coli* which are sufficient for it to be included in the same family". See page 8, lines 6-11, of the instant specification. The protease which is rendered non-functional in the teachings of Vos is a serine protease which meets this definition. Accordingly, the teachings of Vos anticipate the instant claims. Vos teaches that the mutant protease can be obtained by various mutations including site-direction mutagenesis, deletions, and insertions.

***Claim Rejections - 35 USC § 103***

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. Claims 11, 13, 14 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dougan et al (WO 91/15572) or Georgiou et al (US 5,264,365) in view of Smeds et al. (J.Bacteriol. Dec.1998. 180(23): 6148-6153).

Dougan et al teach a method of expressing a heterologous antigen in a bacterial strain which is an htrA mutant, i.e., one that cannot express a functional htrA protease. See page 7, first

full paragraph. It is specifically taught that an expression cassette may be used to produce the htrA mutant bacterial strain. See page 7, second full paragraph. Georgiou et al teaches teach protease-deficient *E.coli* hosts which when combined with an expression system are useful for the production of proteolytically sensitive polypeptides. See abstract. Georgiou et al also teaches that the use of an inducible expression system with a constitutively protease-deficient bacterial host strain was known in the art to solve the problem of low polypeptide production in bacterial host cells.

However, Dougan et al and Georgiou et al do not particularly exemplify the use of a strain of a mutant *Lactobacillus* or other Gram positive bacterium with a bacterial genome size of equal to or less than 3.2Mb

which does not express a functional HtrA protease.

Smeds et al teach a strain of *Lactobacillus helveticus* which does not produce a functional HtrA protease. Smeds et al created a mutant *L.helveticus* by replacing the 5' end of *htrA* with the *gusA* reporter gene. The fusion of the *gusA* gene was downstream of the stress-inducible *htrA* promoter and disrupted the *htrA* gene. See page 6150, column 1.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the *L.heleviticus* mutant which does not express a functional HtrA protease as taught by Smeds to produce a protein of interest because the use of bacterial strains which have disrupted HtrA genes or other genes which encode proteases were known in the art to be good strains for recombinantly producing proteins of interest because the mutants strains will

not produce proteins which will degrade the desired proteins. Both Dougan and Georgiou teach that it was well known in the art to use protease mutants as host cells for producing proteins of interest because they will not act upon polypeptides to effect degradation and because the expression of heterologous proteins is likely to be more favorable in htrA mutants because of the increased stability of recombinant antigens associated with the degP phenotype (see Dougan page 7, first full paragraph). One of ordinary skill in the art would be motivated to use the strain of Smeds for recombinant protein production because they would be expected to get a greater protein yield in the protease mutants and encounter more stability and it would appear to be functionally equivalent to other protease mutant bacterial strains. The Smeds strain would be particularly useful for producing proteins which are native to *Lactobacillus*.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer E. Graser whose telephone number is (571) 272-0858. The examiner can normally be reached on Monday-Friday from 7:00 AM-4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lynette Smith, can be reached on (571) 272-0864.

J Graser 2/24/05

JENNIFER E. GRASER
PRIMARY EXAMINER